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LETTERS TO THE EDITOR

The Theory of Inter-allelic Complementation

It is now almost certain that the genetic phenomenon of inter-allelic complementation is due to the interaction of protein subunits; a partially functional aggregate is produced from two distinct types of subunits neither of which can, by itself, give rise to any appreciable enzyme activity. An adequate theory must show why complementation maps are approximately one-dimensional and why in many cases they are not strictly co-linear with the corresponding genetic maps. We are aware of only one publication concerned with this problem, a theoretical model for complementation in the enzyme adenylosuccinic synthetase (Kapuler & Bernstein, 1963). We present here a sketch of a different general theory of complementation (previously circulated privately, 1960).

The basic idea that complementation is due to protein–protein interaction appears to have been first suggested by Brenner (1959) and Fincham (1960). Recent papers dealing with *in vitro* complementation are Woodward (1959), Loper (1961), Schlesinger & Levinthal (1963), Fincham & Coddington (1963) and Perrin (1963). Reviews covering the subject are by Fincham (1960) and Catcheside (1960).

TABLE 1

	A	B	C	D
A	0	+	0	0
B	+	0	0	0
C	0	0	0	+
D	0	0	+	0

The pattern of complementation expected for defects which compensate each other locally. The defect of mutant A compensates for that of B. Similarly for the pair C and D. + implies that complementation occurs, i.e. that some active enzyme is formed. 0 implies no complementation, i.e. no active enzyme is formed.

The first explanation of complementation that suggests itself is that the defects in two subunits mutually correct each other. Thus a defect, A, in one monomer might, after aggregation, lie next to a complementary defect, B, in the other so that, although the combinations (A+A) and (B+B) both prevent the enzyme from working, in the combination (A+B) the two defects compensate for each other and active enzyme is formed. This may occur from time to time,† but it seems unlikely, for a number of reasons, to be the usual explanation of complementation. In particular, it predicts the one case that is seldom found. If we have two other compensating faults, C and D, then the pattern of complementation would be that shown in Table 1. This leads to the "map" shown in Fig. 1, which seldom occurs. It seems unlikely, therefore, that complementation is generally due to the mutual *local* correction of the effects of two mutations.

† A similar phenomenon, specific suppression within a single peptide chain, has been reported by Helinski & Yanofsky (1963).

We are thus driven to what is in any case a more likely hypothesis, that complementation is usually due to the correction of the misfolding of one monomer (produced by the mutation) by some unaltered part of the other monomer. In other words, not that "two blacks make a white" but that "good corrects bad". We now proceed to develop this idea.

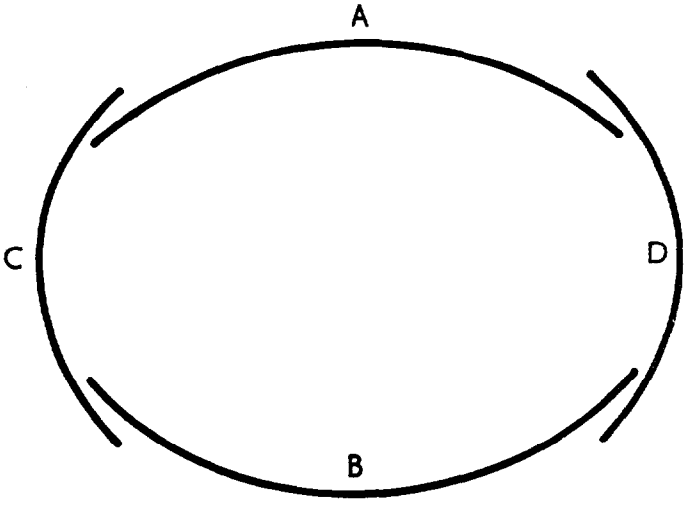

FIG. 1. The complementation map resulting from the complementation pattern of Table 1. This map is circular, not linear. Most sets of any four complementing mutants are found in practice to give a linear map.

For simplicity, we deal only with cases in which the wild-type multimer† is made up of monomers of only one kind; that is, of only one type of polypeptide chain, controlled by a single cistron. Now it is highly likely on general grounds that such an aggregate will have some symmetry. Although we cannot completely rule out fancy pseudo-symmetries (e.g. a shallow screw axis terminated by steric hindrance), by far the most likely symmetry elements are axes of rotation. True mirror planes and centres of symmetry are of course forbidden because proteins consist only of L-amino acids. Thus we may confidently expect that most multimers will have n-fold rotation axes, often of more than one type. Twofold axes are likely to be most common.

We consider first the special case in which all monomers are inactive because the active site is made up of parts of two (or more) subunits. This might, for example, occur when two monomers form a dimer as shown diagrammatically in Fig. 2.

In this case we would have complementation between two monomers, one of which had an inactive region *a*, and the other an inactive region *b*. Thus in Fig. 2 the right-hand site would be inactive, but the left-hand site would function. This may be the correct explanation in some cases, but it can hardly provide a basis for a general theory of the approximate linearity of complementation maps spreading over considerable lengths. Note, in passing, that complementation, as in this example, may often lead only to a *partial* restoration of activity.

For the general theory we shall make no special assumptions about the activity of monomers, nor about the location of the active centre.

† We have used the term "multimer" to denote an aggregate of several (identical) objects without any implications as to their geometrical arrangement; we prefer to retain the word "polymer" for systems in which the aggregation is linear and involves small molecules held together by chemical bonds.

Our basic structural assumptions are:

(1) that certain mutations can produce a local misfolding of the protein, which in its turn prevents enzyme activity;

(2) that this can be corrected by interacting with an adjacent region which has the correct (wild-type) fold.

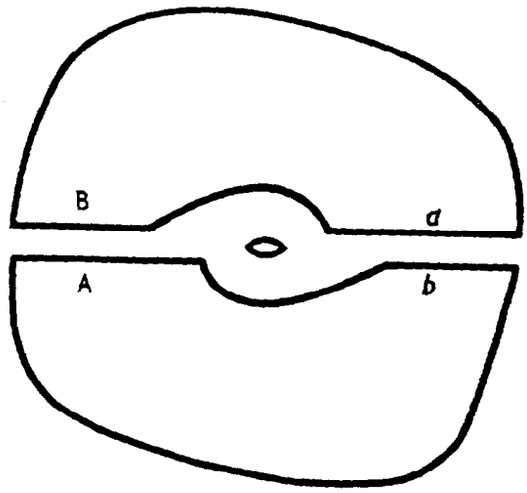

FIG. 2. A very diagrammatic illustration of an enzyme which is a dimer and only forms the active site when the parts marked A and B come together. The Figure shows the mixed dimer formed by two complementing mutants. The upper molecule is defective at site A (marked therefore as *a*), the lower one at B (marked as *b*). Thus the site on the right is defective while that on the left is still active.

These assumptions are in essence the same as those of Kapuler & Bernstein (1963). Since a study of myoglobin has shown (J. C. Kendrew, personal communication) that the stability of proteins depends largely on side-chain interactions between stretches of the polypeptide chain which are adjacent to each other (i.e. on interactions between amino acid side chains on *neighbouring* helices, rather than those on the same stretch of helix), these assumptions are plausible.

Now consider some change of an amino acid, produced by a mutation. This can have several effects.

(1) It may not significantly alter the folding or, if it does, the activity of the enzyme may be unchanged. In this case it will not normally be picked up as a mutant.

(2) It may destroy the local folding in such a way that the adjacent, unaltered regions of the polypeptide chains, either in the monomer or in the multimer, cannot correct it. In this case, if the misfolding destroys the activity of the enzyme, it will appear as a non-complementing mutant.

(3) It may destroy the local fold of a monomer, but this may be correctly refolded by an unaltered adjacent non-homologous region of one of the *other* polypeptide chains in the multimer. In this case it would not normally be picked up as a mutant, since, although the monomer of the homozygous mutant is damaged, the multimer is still functional.

Thus, none of these cases will lead to complementation. To achieve complementation the misfolded region must lie so that it can only be corrected by part of the *homologous region* (correctly folded) in one of the other monomers of the multimer. It is easily seen that this is most likely to occur *in the regions adjacent to the axes of rotation* of the multimer.

This basic prediction can easily be seen to lead to the general type of relationship observed in complementation. Mutants affecting the same region, near a rotation axis, will often be near together on the genetic map. Thus in many cases such mutants will not complement each other. The length of a "segment" in a complementation map will be a rough measure of the length of the region misfolded by the mutants in that segment. If misfolding spreads along the length of the polypeptide chain there will be a general tendency for the genetic map and the complementation map to be co-linear, but there will be many exceptions. If it spreads not along the chain but to adjacent folded segments, the complementation map may remain linear but it may not be co-linear with the genetic map. Complicated misfoldings may easily produce non-linear, circular or spiral complementation maps.

We have considered:

(1) the effect of triad and higher axes of symmetry;

(2) the possibility that some mutants may cause the production of a fragment of the polypeptide chain;

(3) the efficiency of complementation in the various cases. It suffices to say that this has not led to anything which contradicts our basic idea.

We disagree with the suggestion of Kapuler & Bernstein (1963) that because complementation maps are relatively simple "there exists a class of enzyme . . . whose *in vivo* tertiary configuration is simple, and that in at least one case this configuration is approximately that of a two-turn spiral". In our view these authors have not clearly realized that the requirements for mutants to complement are rather special and that symmetry axes are likely to be involved. Moreover, their suggestion that protein subunits are likely to aggregate into a layered stack is implausible. Complementation is a very common phenomenon and special explanations are unlikely to be correct.

We believe, as they do, that the details of the folding of the polypeptide chain are the key to the relationship between the complementation map and the genetic map, but we do not believe that there is any *simple* general way of deducing these details from the supposed correspondence between the two maps.

It must always be realized that while the genetic map is a linear map relating point mutants, the complementation map consists of segments and does not put the point mutants into a unique order. Thus one always has a certain amount of latitude in comparing the two kinds of maps, and a good deal of discretion is therefore required in assessing the success of a theoretical prediction.

Although we believe that the general theory of complementation we have sketched here is plausible, it can hardly be accepted as more than a useful working hypothesis without further experimental evidence. Unfortunately, the structure of proteins is so complex that we have been unable to devise any crucial experimental test of our theory which does not depend on an *a priori* knowledge of the configuration of the

protein. Such information is obtained only from lengthy X-ray studies. We believe that it is essential for any detailed test of our general theory or, indeed, of any other theory of complementation.

We should like to thank the numerous workers in the field who commented on our original manuscript on this subject, and in particular Dr. Chester Partridge who sent us many detailed criticisms. The basic idea originally came to us after conversations with Dr. Norman Giles, to whom we are particularly grateful for instructing us on the facts then available.

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